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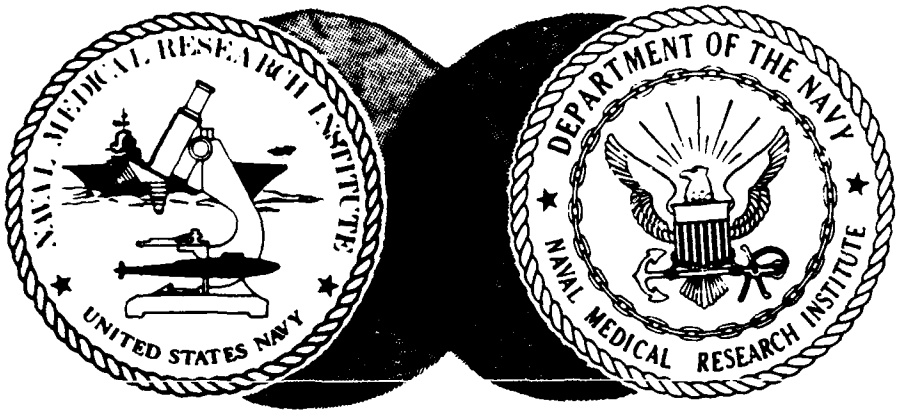

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EXPERIMENTAL MANDIBULAR OSTEOMYELITIS: THERAPEUTIC TRIALS WITH HYPERBARIC OXYGEN

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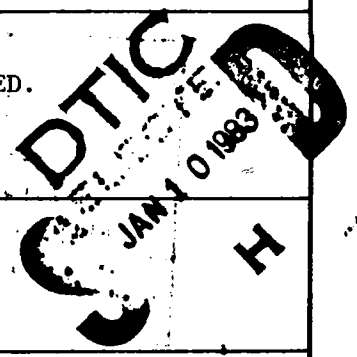

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Hyperbaric oxygen (HBO) has been reported to be beneficial in the treatment of mandibular osteomyelitis; however, controlled laboratory studies have been limited to the long bones. In this study, osteomyelitis was created in surgically fractured rabbit mandibles by inoculation of Bacteroides melaninogenicus. Two months after inoculation, osteomyelitis was verified by bacterial cultures and inspection of the fracture sites. The animals were then randomly divided into treatment and control groups. The treatment group received HBO (2 atmospheres) for two hours daily for 40 treatment days, whereas the control

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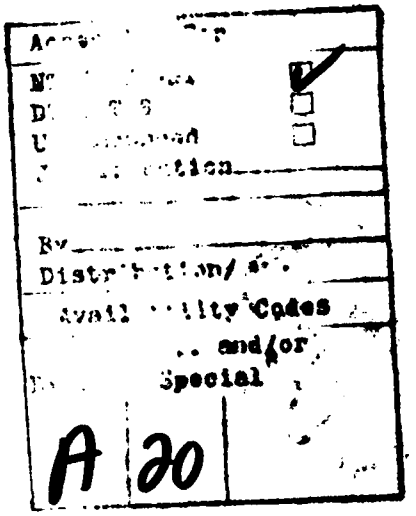

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Experimental Mandibular Osteomyelitis:
Therapeutic Trials with Hyperbaric Oxygen

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Hyperbaric oxygen (HBO) has been reported to be beneficial in the treatment of mandibular osteomyelitis; however, controlled laboratory studies have been limited to the long bones. In this study, osteomyelitis was created in surgically fractured rabbit mandibles by inoculation of *Bacteroides melaninogenicus*. Two months after inoculation, osteomyelitis was verified by bacterial cultures and inspection of the fracture sites. The animals were then randomly divided into treatment and control groups. The treatment group received HBO (2 atmospheres) for two hours daily for 40 treatment days, whereas the control group was maintained on ambient air. Although HBO therapy did not eliminate the chronic osteomyelitis, it did result in a significant improvement in sinus tract healing, osseous repair, and diminished mobility at the fracture site.

Osteomyelitis frequently causes management problems for the clinician in spite of the use of potent antibiotics and aggressive local treatment.[1] Presently, the recommended treatment consists of (1) incision and drainage of the infected area (at which time specimens are obtained for culture and antibiotic sensitivity testing), (2) large parenteral doses of appropriate antibiotics, (3) sequestrectomy, (4) frequent irrigation of the area through drains, and (5) supportive care.[2] Regardless of how meticulously these treatments are performed, the disease often fails to subside. This failure may result from compromised vascularity of the affected tissues, which prevents oxygen, antibiotics, and nutrients from reaching the diseased area in adequate concentrations for wound repair.[3]

Hyperbaric oxygen (HBO) therapy as an adjunctive treatment for mandibular osteomyelitis has been reported to be clinically beneficial,[4,5] although controlled studies in experimental animals have been limited to the long bones.[6,7] This treatment is also well established for gas gangrene, decompression sickness, gas embolism, and carbon monoxide poisoning.[8] More recently, it has been used for burns,[9] hypoxic soft-tissue wounds,[10] cerebral edema,[11] mandibular osteomyelitis,[4,5] and osteoradionecrosis.[12,13,14]

The rationale for the use of HBO in the treatment of osteomyelitis is based on favorable events that occur when the partial pressure of oxygen (P_{O_2}) is raised. These include increases in capillary budding, osteoclastic and osteoblastic activity to remodel bone, callus formation and mineralization, and bactericidal activity of leukocytes in the wound.[15]

The purpose of this study was to determine whether HBO therapy would improve osseous repair in an experimental animal model of mandibular osteomyelitis.

Material and Methods

Mandibular fractures were surgically created in 32 male New Zealand white rabbits weighing 4 to 5

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The opinions and assertions contained herein are the private ones of the authors and are not to be construed as official or reflecting the views of the Navy Department or the naval service at large.

The experiments conducted herein were conducted according to the principles set forth in the *Guide for Care and Use of Laboratory Animals*, Institute of Laboratory Resources, National Research Council, DHEW, Pub. No. (NIH) 78-23.

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kg. The rabbits were anesthetized by intramuscular injection of a combination of 25 mg ketamine hydrochloride and 0.25 mg acepromazine hydrochloride per kg body weight. Each animal was placed in the supine position, and the left mandibular region was shaved, washed repeatedly with surgical soap, and covered with sterile drapes for an extraoral procedure. The soft tissues and the periosteum overlying the left side of the mandible were infiltrated with 3.6 ml of 2% lidocaine hydrochloride with epinephrine 1:100,000 to supplement the general anesthetic and for hemostasis.

The body of the left half of the mandible was surgically exposed from the symphysis to the ramus. By use of a bone bur, vertical cuts approximately 2 cm apart were made through the cortical bone on the medial and lateral surfaces. These cuts were connected by a linear osteotomy on the inferior border of the mandibular body (Fig. 1). A sagittal fracture was then produced by use of an osteotome. The medullary bone was cauterized with a heated spatula to compromise the local blood supply and to provide a coagulum for bacterial growth. A 1 × 1 × 0.7 cm piece of Gelfoam was inserted between the cortical plates, which were loosely approximated with 0.02 inch stainless steel wire. The enclosed Gelfoam was then injected with 0.25 ml of an inoculum containing approximately 10^7 bacteria per ml. The wound was immediately closed without drains by suturing the subcutaneous tissue with 4-0 chromic gut and the skin with 4-0 nylon. The bacterial inoculum was a pure culture of the synergistic oral pathogen *Bacteroides melaninogenicus*.[16]

Postoperatively, the animals were housed in stainless steel controlled-environment isolation chambers and fed a leafy vegetable diet and water ad libitum. They were maintained in this manner for eight weeks to allow time for the infection to become chronic. Four animals died during this period. At the end of the eight-week incubation period, the surviving 28 rabbits were reanesthetized and the fracture sites were surgically exposed and evaluated for osteomyelitis. The following criteria were required for that diagnosis: (1) an obvious osseous lesion, (2) purulent exudate from the wound with or without draining sinuses, (3) mobility of the fractured fragments, and (4) isolation of bacteria from the fracture site. Bacterial cultures and Gram stains were performed on material from the bony lesions. The specimens for culture were immediately added to tubes of peptone-yeast-glucose broth, which were then placed in an anaerobic atmosphere of 85% N_2, 10% CO_2, and 5% H_2. Direct platings were made to brain-heart infusion agar plates supplemented with vitamin K and heme for isolation of obligate anaerobic microorganism in-Vancomycin

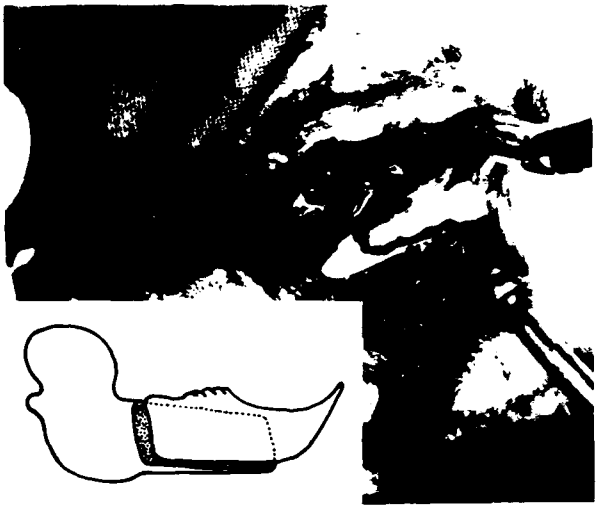

FIGURE 1. View of mandibular osseous cuts involving lateral surface and inferior border before sagittal fracture. *Insert*, Schematic of the cuts.

laked blood agar plates were also used in the anaerobic atmosphere. These antibiotic-treated plates were used to prevent overgrowth of facultative microorganisms and thereby facilitate the isolation of obligate anaerobic microorganisms. Cultures were also made in ambient air on 5% sheep blood agar plates for isolation of aerobic and facultative microorganisms. These plates were incubated in a 10% CO_2-enriched atmosphere. All isolates were identified by API 20E* and Minitek† systems in conjunction with gas liquid-chromatography procedures for obligate anaerobes.[17]

After it had been verified that each rabbit satisfied all the criteria for osteomyelitis, the animals were randomly divided into treatment and control groups. In the treatment group, 16 rabbits received 100% O_2 at 2 ATA (1520 mm Hg) for two hours daily, five days a week, for a total of 40 treatments (80 HBO hours). This treatment began nine weeks after bacterial inoculation. HBO was administered to groups of four animals in a hyperbaric chamber. During treatments, the rabbits were separated in a compartmented custom-made cage to minimize excitement and movement. The O_2 concentration was kept between 98 and 100%, and the CO_2 level below 0.1%. The control group, 12 animals, breathed ambient air at normal atmospheric conditions (760 mm Hg).

Hematocrit (Hct) determinations, leukocyte counts, and reticulocyte counts were performed five days prior to bacterial inoculation and at one, three, five, and eight weeks during the eight-week incubation period. During the subsequent HBO treatment period

* Analab Division, Amherst Laboratories.
† Baltimore Biological Laboratories and Falcon Products.

Table 1. Microorganisms Isolated from Lesions of Mandibular Osteomyelitis

	Aerobes and Facultative Anaerobes					Obligate Anaerobes			
	No. of Isolates in HBO Treated Group		No. of Isolates in Control Group			No. of Isolates in HBO Treated Group		No. of Isolates in Control Group	
Microorganism	(8 wk)	(17 wk)	(8 wk)	(17 wk)	Microorganism	(8 wk)	(17 wk)	(8 wk)	(17 wk)
Staphylococcus aureus	5	4	3	2	*Bacteroides* sp	4	3	1	1
Staphylococcus albus (nonhemolytic)	5	3	2	3	*Bacteroides melaninogenicus*	2	0	1	0
Proteus sp	4	3	1	2	*Clostridium* sp	4	2	1	2
Escherichia coli	4	7	3	4	*Peptostreptococcus* sp	1	0	1	0
Hemophilus sp	3	3	1	1					
Streptococcus sp	3	3	2	2	Total isolates	11	5*	4	3
Neisseria sp	1	1	0	0					
Diphtheroid sp	1	0	0	0					
Pseudomonas sp	0	0	1	0					
Aerobacter sp	0	0	1	0					
Total isolates	26	24	14	14					

HBO Treated: N = 16
Control: N = 8
* Bacterial overgrowth by facultative organisms prevented isolation of obligate microorganisms obtained from four animals at 17 weeks in the HBO treated group.

(nine to 17 weeks after bacterial inoculation), these studies were repeated at 10, 12, 14, and 16 weeks.

At the end of the HBO treatment period, all animals were anesthetized to determine the presence of draining sinuses and to obtain the final bacterial cultures. Immediately afterward, they were killed by bilateral carotid artery perfusion with formaldehyde-glutaraldehyde fixative.[18] The lungs were perfused through the trachea with the same solution. Specimens of brain, lung, and eyes were collected from all rabbits for microscopic evaluation of oxygen toxicity.

The mandibles were removed and divided at the symphysis, and radiographs of both halves of each mandible were made to assist in the evaluation of osseous destruction and repair. On the basis of the extent of radiopacity, the radiographic interpretation of healing was scored as well healed, moderately healed, or minimally healed. The soft tissues were removed, and osseous defects and fracture mobility were documented. Mobility of the fractured region was subjectively categorized as gross, moderate, or minimal. Mobility was also quantitatively evaluated by fixing the distal portion of the fractured left half of the mandible and recording movement of the proximal portion with an indicator gauge in response to a 1000 gram force applied by means of a dynamometer to the medial and then the lateral sides. If movements were not equal in both directions, the greater measurement was recorded. Fracture mobility was categorized as: >0.6 mm, between 0.6 and 0.06 mm, and <0.06 mm. Specimens from the fractured left half of the mandible of all animals were prepared for histologic examination. In addition to hematoxylin and eosin staining, the Brown and Brenn staining method[19] was used to identify the presence of bacteria in the five mandibles (three treated and two control) that had the greatest mobility. All slides were examined by three observers who were unaware of the category of each specimen.

Statistical analysis was performed by (1) chi square for the mobility scores, (2) paired or unpaired Student's t test for the incidence of sinus tracts and hematologic and microbiologic data, and (3) correlation coefficients for comparison of the radiographic and mobility scores.

Results

A chronic suppurative osteomyelitis of the mandible was consistently produced in this animal mode. Although every fracture was initially infected with a pure inoculum of *Bacteroides melaninogenicus*, subsequent cultures demonstrated a mixed bacterial flora (Table 1). Preliminary studies had indicated that obligate anaerobic bacteria could be eliminated by 80 hours of HBO treatment. However, in the present study, obligate anaerobic, facultative anaerobic, and aerobic isolates were identified in both control and treated groups at the end of the HBO treatment period. There was no statistical reduction in isolates of aerobic, facultative, and obligate anaerobic microorganisms in the treated compared with the control animals ($P > 0.50$), and osteomyelitis was not eliminated in any of the rabbits. In four of the specimens obtained from the treated animals, overgrowth of the obligate an-

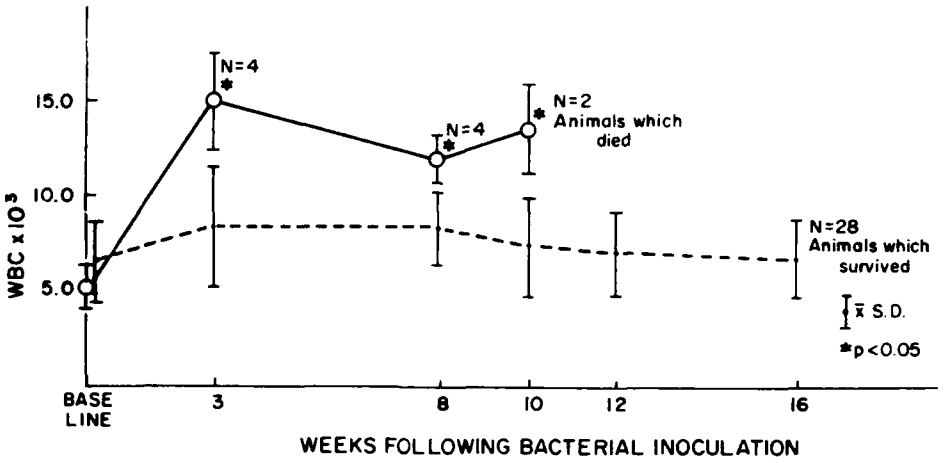

FIGURE 2. Comparison of the mean leukocyte counts between the animals which died during the experiment and those which survived. N represents the animals alive at the sampling periods indicated.

aerobes by *Proteus* species prevented isolation and identification of those bacteria.

Eight of 32 animals died during this study. Four died during the eight-week incubation period. Necropsy and culture of these four animals showed a systemic *Pasteurella multocida* infection. Four additional animals that had been assigned to the control group died during the HBO treatment period, two of systemic *P. multocida* infections and two of unknown causes. There were large elevations of the leukocyte counts (≥16,000/cu mm) in the animals that eventually died. These were significantly greater than in the animals that survived ($P < 0.05$) (Fig. 2).

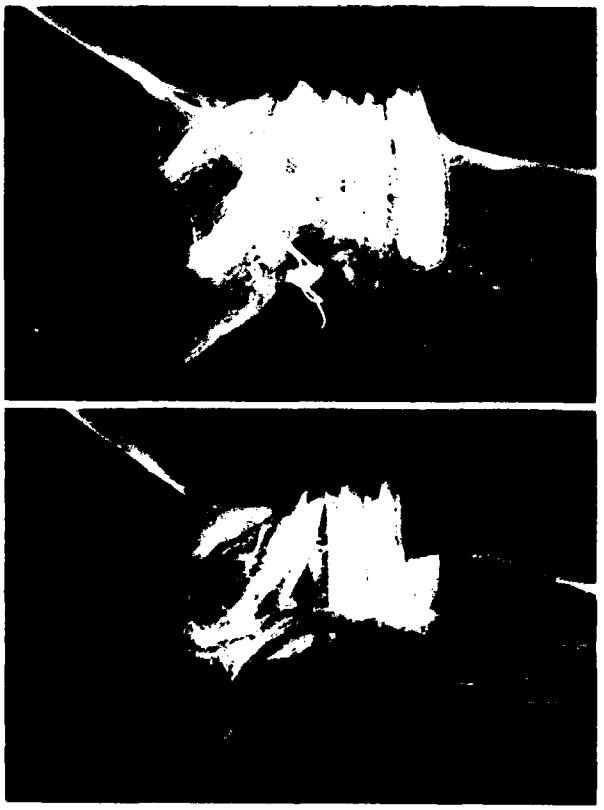

FIGURE 3. *Above*, Radiograph of operated left side of the mandible, showing osseous defects, fractured tooth roots, and sequestra. This specimen was stable.

FIGURE 4. *Below*, Radiograph of operated left side of mandible of another animal, showing features similar to those in Figure 3 but with a larger area of bony destruction. The specimen was nonetheless stable.

Following bacterial inoculation, the mean leukocyte counts were significantly increased above baseline in both the treatment and the control groups ($P < 0.05$). The mean leukocyte counts did not differ significantly from baseline values at the 12- and 16-week samplings in the treatment group and at 16 weeks in the control group. There were no significant differences in the mean leukocyte counts between the two groups at any of the sampling periods. The reticulocyte counts were significantly decreased in the treatment group only at the eight-week sampling, but those for the controls were unchanged throughout the period of observation. In both groups the hematocrits remained similar to the baseline values throughout the experiment.

By the end of the experiment, draining sinuses had been eliminated in five of six HBO-treated animals but in only one of four controls. This difference was statistically significant ($P < 0.05$).

Subjectively, in 11 of 16 (68.8%) HBO-treated animals the fractures were deemed stable; two had moderate mobility and three gross mobility. In the control group only one of eight (12.5%) fractures was stable; five were moderately mobile and two were grossly unstable ($P < 0.02$). By quantitative mobility measurements, 13 mandibles (12 treated [75%] and one control [12.5%]) were determined to be stable. Five control mandibles and one treated mandible had between 0.06 and 0.6 mm of movement, and two control and three treated mandibles had more than 0.6 mm of movement. The quantitative difference in stability between the two groups

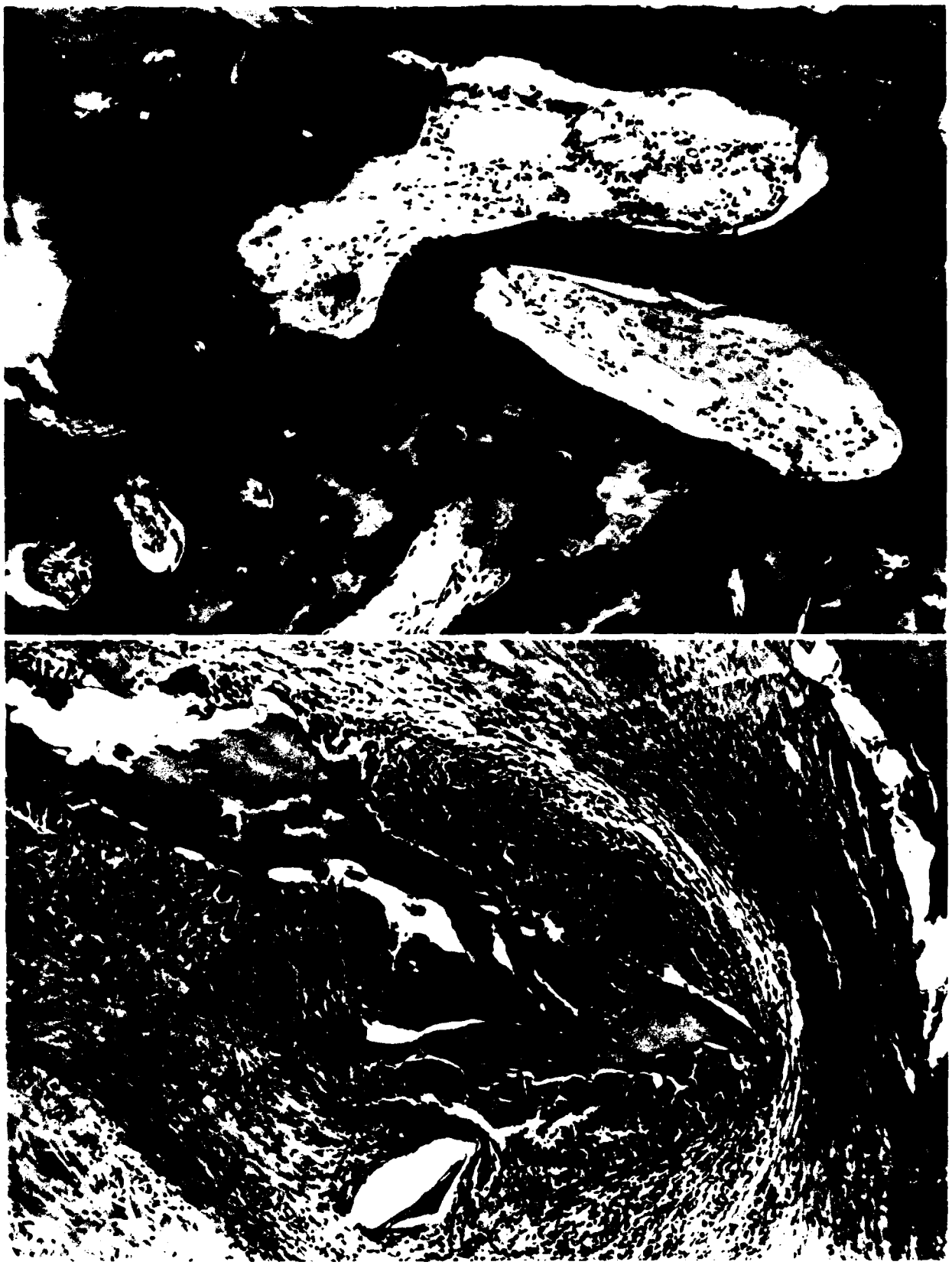

FIGURE 5. *Above*, Section from a stable mandible showing well-organized osseous repair (hematoxylin and eosin, ×200).
FIGURE 6. *Below*, Bony sequestrum surrounded by chronic inflammatory cell infiltrate and dense connective tissue typical of the histologic pattern seen in specimens from unstable mandibles (hematoxylin and eosin, ×200).

was also statistically significant ($P < 0.01$). The subjective and quantitative assessments of stability were thus in good agreement.

By use of lateral mandibular radiographs alone to assess healing, seven treated and three control mandibles were judged to be well healed; five treated and three controls moderately well healed; and four treated and two controls poorly healed. The assessment of osseous repair and fracture stability based on interpretation of a single radiograph often did not parallel the subjective and direct quantitative measurements of mandibular stability (Figs. 3 and 4).

Active osteogenesis and repair, with ingrowth of connective tissue and capillaries, were noted histologically in all mandibles deemed to be stable. In contrast, the unstable mandibles had dense leukocytic infiltration, sequestra formation, and absence of osteoblastic activity. Figures 5 and 6 show the histologic patterns in one stable and in one unstable mandible. The five mandibles with the poorest stability contained gram-positive bacteria in one of three treated specimens and in both of the controls. No gram-negative organisms were observed, although they were identified in bacterial cultures from two (one treated and one control) of these five mandibles.

Microscopic examination of brains, lungs, and eyes of all rabbits showed no appreciable differences between the treatment and control groups. Histologic changes indicative of pulmonary O_2 toxicity (i.e., increased pulmonary fibrosis, hemosiderosis, or extensive edema)[20,21] were not observed by either light or electron microscopy.

Discussion

Although the infection was not eliminated in any of the animals, as evidenced by bacterial isolates and persistence of osseous lesions, HBO therapy did improve osseous repair and fracture stability. The more rapid return of the leukocyte count to baseline values in the HBO treatment group suggested that the infection was better controlled in this group than in the controls. A leukocyte count $\geq$16,000/cu mm was a poor prognostic sign because the animals with counts this high eventually died. In the surviving animals of both groups it appeared that the host defense mechanisms were able to localize the infection, allowing the leukocyte counts to return to baseline values. A decrease in the number of draining sinuses indicated that healing of soft tissue wounds was also enhanced by HBO therapy. This observation is similar to that reported by Hamblen.[6]

These results may have been due in part to an enhanced host defense mechanism. Adequate tissue oxygen tension is essential for effective leukocyte killing of certain bacterial pathogens.[22] The increase of P_{O_2} in a hypoxic wound (e.g., osteomyelitic lesion) produced by HBO treatment may enhance leukocyte killing and improve the local environment, favoring neovascularization, epithelization, collagen synthesis, and eventual osseous repair.

Another mechanism by which HBO may enhance healing is a direct inhibitory effect on certain bacterial flora. Nuckolls and Osterhout[23] reported that HBO (3 ATA for two hours, twice a day) inactivated *Bacteroides* species grown both in vitro and in vivo, and Gottlieb[24] described oxygen as having broad-spectrum bacteriostatic effects. Because of these reports, an inhibition of bacterial growth was expected. However, we were unable to eliminate the bacterial infection with the HBO regimen employed. It is possible that its effectiveness may have been disguised because the compound fractures communicated with the oral cavity and allowed continual bacterial repopulation of the wound, either by direct spread or through secondary contamination by transfer of organisms from the cages and from other areas of the body by licking. Additionally, the 2 ATA of HBO therapy once a day may not have provided sufficient oxygen to inhibit bacterial growth. Employing the HBO treatment at 3 ATA twice a day, as reported by Nuckolls and Osterhout,[23] might have inhibited the bacterial growth. Their dosage schedule was not used in this study because we chose to simulate the HBO exposures utilized in clinical trials. In clinical trials, the HBO exposures are usually limited to 2.0 to 2.4 ATA because of the possibility that oxygen toxicity may develop. Oxygen toxicity is nonspecific and can affect all tissues of the body if sufficiently high concentrations are used; however, the lung usually provides the first indication that toxicity is occurring because of its exposure to the high partial pressure of inspired oxygen.[20]

Pathologic changes in the lung resulting from oxygen toxicity consist of atelectasis, edema, alveolar hemorrhage, inflammation, fibrosis, and hyalinization of the alveolar membrane.[20] In clinical use, breathing 100% O_2 at 2.0 to 2.4 ATA for two hours daily, five to six days a week for 40 treatments, has not produced any of the major complications of oxygen toxicity.[1,25] Likewise, in this animal model we were unable to detect such signs following similar exposures. At higher pressures and extended exposures, hemolysis and anemia have been reported,[26] as well as depression of erythropoiesis.[27] These complications, however, have not been seen in our studies or in clinical practice.[25,28] It was not apparent why, eight weeks after

inoculation, the reticulocyte counts were slightly depressed in just the treatment group, because all animals were similarly treated during this period. The mean reticulocyte counts in the treatment group were otherwise similar to baseline values and to those in the untreated controls. This supports the clinical findings that, in man, erythropoiesis is not depressed by intermittent HBO treatment at 2.0 to 2.4 ATA.[25,28]

The subjective and quantitative assessments of stability paralleled the histologic findings. However, the correlation with the radiographic findings was poor. Perhaps it would have been desirable to obtain additional radiographic views in order to see whether the relationship could have been improved.

The aim of HBO therapy is to improve tissue P_{O2} in a hypoxic wound, thereby enhancing vascular proliferation, fibroblastic activity, and collagen formation.[13] These events are prerequisites for osseous repair, and their enhancement may account for the increased fracture stability and osseous repair found in the HBO-treated animals.

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